Genotoxic Effect of Cadmium on Mung Bean Seedlings and its all Eviation by Priming with Turmeric Extract

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Abstract

Pollution is the major problem in all over the world and it made negative impact on all crops and human health. There are many reasons like industrial wastes and various anthropologic activities for metal toxicity in river, canals and so to agricultural lands, air and drinking water. Cadmium (Cd) contamination is very severe and causes DNA alterations and may become toxic and carcinogenic when consumed by human. Turmeric has been found to have antioxidant activity and used as medicinal herb for many decades. Mung bean was used as test organism to corelate the effect of cadmium on human. This investigation reveals that the toxic effect of Cd on mung bean could be alleviated by priming with turmeric extract. Cd was also known to reduce growth of plants and can cause various chromosomal aberrations. In cytogenetic analysis, we found that pretreatment of turmeric induced the anti-mutagenic effect in response to Cd in mung bean root tips by showing reduced mitotic index (MI) and total chromosomal aberrations. As priming with turmeric extract prior to cadmium stress reduces MI and growth of mung bean seedlings with lesser chromosomal aberrations, it can be used as a compound to reduce the growth of tumor as well.

Keywords:

Cadmium, Chromosomal Aberrations, Curcumin, Mung Bean, Turmeric.

1. INTRODUCTION

Modern world requiring modern techniques and elements which can be hazardous for all living systems. These elements are now getting involved commonly in the rivers, canals and agricultural land. Specially the industrial waste that include the heavy metals like Pb, Cd, Cu, Ti, Ni and many more. If any living body including plants and animals get expose to such heavy metals, will destroy the functional mechanism and can lead to lethality. The most common and toxic metal present among such heavy metals is cadmium

(Cd) because of its inherent ability to cause many changes in plants and animals, including proteins and DNA alterations. Cadmium can be accumulated in the soil by extensive use of phosphate fertilizers that contain cadmium as contamination. Cadmium is a highly toxic pollutant to prokaryotic and eukaryotic organisms also due to its solubility in water, which determines a rapid distribution in the environment. Uptake of Cd by crop plants is the main entry pathway into the food chain causing serious problems to human health (Buchet *et al.,* 1990) like cardiovascular, renal, neurological, reproductive, gastrointestinal, and respiratory systems disorders.

Plant materials may use as test system that can well explain the mechanism in human. In crop species like bean and wheat, cytotoxicity of Cd exposure appears as chromosomal aberrations and inhibition of mitotic processes with consequent altered cell cycle and division (Benavides *et al.,* 2005), poor seed production and malformed embryos (Ernst *et al.,* 2008). Cd resistance involves the synthesis of stress-related proteins and signal molecules (heat shock proteins, salicylic and abscisic acids, ethylene) (Sanit 'a di Toppi and Gabbrielli 1999).

The use of herbs for the treatment of various diseases including cancer has gained an importance nowadays. These herbs are natural antioxidants to cope up with diseased oriented oxidative stress by scavenging free radicals and modulating antioxidant defense system (Salama and El-Bahr, 2007) Turmeric is widely used as medicinal herb that has an active component, curcumin. Turmeric powder is used extensively as a colouring and flavouring agent in curries and mustards. Turmeric is one of the most popular medicinal herbs, with an extensive range of activities such as water and fat-soluble extracts of turmeric and its curcumin component exhibit strong antioxidant activity analogous to vitamins C and E (Toda *et al.,* 1985), anti-protozoal, anti-venom, anti-microbial, anti-inflammatory as it reduces inflammation by lowering the levels of histamine and possibly by increasing the production of natural cortisone by the adrenal glands (Ammon *et al.,* 1993). It is reported to cause anti-angiogenic, anti-tumor arrests of carcinomatous cells during the G2 / M process of the cell cycle, where cells are more vulnerable to cytotoxic radiotherapy effects (Wilken *et al.,* 2011) and anti-aging (Amalraj *et al.,* 2016). Curcumin has the antimutagenic ability of clastogenic damage caused by sodium azide in *Allium cepa*. Dose-dependent, but found effective at low doses (Ragunathan *et al.,* 2007). Curcumin not only exhibits antioxidative and free radical scavenging properties, but also enhances the activities of other antioxidant enzymes. Kim *et al.,* 2012 has demonstrated that turmeric is more effective at inhibiting the growth of breast cancer cells than curcumin alone (66 percent growth inhibition). The present study is an attempt to observe the alleviating role of 5 µg/mL turmeric extract pretreatment on cadmium stressed mung bean seedlings. Mung bean will act as model system that would reflect the effect on other plants as well as on animals and human. Furthermore, it could be an attempt to reduce the progress of cancerous cells by the use of turmeric extract.

2. MATERIALS AND METHODS

Fresh rhizome of turmeric 6.8g was cut into small pieces and homogenized in clean and sterilized pestle and mortar in 100 ml of distilled water (D/W) and boiled for 15 min. The aqueous extract was filtered through Whatman filter paper (Arumai *et al.,* 2018) for the use in our experiment. (Fig 1-2)

Fig. 1 Fresh Tumeric

Fig. 2 Aqueous extract of turmeric

Cadmium acetate (0.5 mM) solution was prepared. Seeds of mung bean variety NM-19-19 were obtained from National Agriculture. (Fig.3).

Fig. 3 Mung bean Seed

Research Center (NARC), Islamabad and were treated with sodium hypochlorite (1%) for 5 minutes and rinsed three times with D/W. Seeds were soaked in two separate beakers containing D/Wand turmeric extract (5 µg/mL) solution for 2 h. The seeds from each beaker were placed on Petri dishes to germinate for 24 h at 30°C. Germinated seedlings were treated with D/W and cadmium acetate solution (0.5 mM) for 3 h separately in such a way that four treatments were prepared; 1). Negative Control (D/W); 2) Positive control (D/W + Cd); 3) Treatment group at Research Center (NARC), Islamabad and were treated with sodium hypochlorite (1%) for 5 minutes and rinsed three times with D/W. Seeds were soaked in two separate beakers containing D/Wand turmeric extract (5 µg/mL) solution for 2 h. The seeds from each beaker were placed on Petri dishes to germinate for 24 h at 30°C. Germinated seedlings were treated with D/W and cadmium acetate solution (0.5 mM) for 3 h separately in such a way that four treatments were prepared; 1) Negative Control (D/W); 2) Positive control (D/W + Cd); 3) Treatment group (Turmeric + D/W); and 4) (Turmeric + Cd). For cytological examination, three root tips/ treatment were cut immediately after cadmium treatment and remaining seedlings were allowed to grow for 10 days to record seedling length (cm).

2.1. Cytological Studies

Germinated seedlings were treated with cadmium solution for 3 hours. Three root tips/ treatment were cut immediately after cadmium treatment with or without turmeric pre-treatment. Slides were prepared from each root tips following Fiskesjo, (1985) squash technique. Mung bean root tip cells were scored 700 per slide. Photographs were captured at 100 X magnification using Nikon camera.

2.2. Statistical Analysis

Data with three replications were analyzed by using MS Excel 2016 and statistical analysis was done by SPSS version 20 subjected to one way

ANOVA in CRD and Duncan test was used for comparing treatment means at pd"0.05 level of significance.

3. RESULTS AND DISCUSSIONS

3.1. Mung bean Seedling Length

The length of 10 days old seedlings was measured in cm. Fig. 4 shows that the mean length of control sample was 12.78 cm. Result showed that the seeds that exposed to Cd (0.5 mM) caused significant growth inhibition (8.01) as compared to control. The priming of mung bean seedlings with turmeric (5 µg/mL) a significant increase in length (13.7 cm) as compared to control suggesting that turmeric promotes the growth of seedlings but when the mung seeds were pretreated with 5 µg/mL turmeric prior to Cd showed significantly decrease in seedling length (3.9).

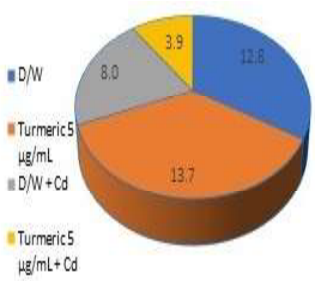

Fig. 4 Mean seedling length after cadmium stress and priming with turmeric prior to Cd

This may be due to anti-angiogenic effect of turmeric in response to Cd as shown in Fig 4. Angiogenesis means the growth of new blood vessels and anti-angiogenic effect of turmeric treatment reflects the inhibition of tumor formation from growing their own blood vessels, this might slow down the growth of cancer or sometimes shrink it.

Fig. 5 exhibited 7% the promotion on the growth of mung seedlings after priming with 5 µg/mL turmeric and later grown in distilled water. The inhibitory effect of cadmium on the growth of that mung seedlings was significantly low (37% inhibition) in cadmium treated seedlings.

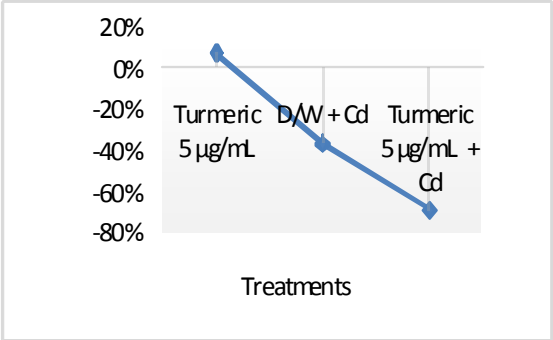

Fig. 5 Percentage promotion / inhibition of seedling length after cadmium and priming with turmeric extract prior to cadmium.

However, turmeric (5 µg/mL) pretreatment prior to cadmium exhibited 69.1% inhibition as compared to control. It is clear that turmeric pretreatment before distilled water was able to enhance the growth, but when applied prior to cadmium stress reduced the growth of mung bean seedlings. It showed that turmeric extract under normal condition is mitogenic but under stressed condition (Cd) it is anti-mitotic.

3.2. *Cytological Analysis*

Table 1 represented the mean sum of square for different chromosomal aberrations observed after cadmium treatment and after pretreatment with turmeric extract. It was observed that there was significant differences between treatments for all aberrations. Table 2 demonstrated that the treatment with Cd caused genotoxic effect by showing reduction in mitotic Index (MI) percent and induction of chromosomal aberrations in dividing root cells. The aberrations observed were sticky chromosomes, lagging chromosomes, fragment formation and C-mitosis, bridges.

The result also showed C-mitosis was most frequent amongst aberrations when mung bean seeds were primed with turmeric than sown in d/w or under cadmium stress.

Table 1. Mean sum of square of normal and abnormal mitotic stages in mung bean roots.

Sources of Variations	df	MS									
		Normal Mitotic Stages				Abnormal Mitotic Stages					
		Prophase	Metaphase	Anaphase	Telophase	St. Meta	Dis. Meta	Laggards	C-Mitosis	Bridges	Fragments
Between treatments	3	10662.7*	2541.45*	4199.3*	453.8*	688.97*	302.6*	420.3*	235.86*	1.47*	206.7*
Within treatments	8	12.66	8.16	11.2	9.417	3.25	1.08	0.917	3.917	0.083	1.66

Table 2 Mean Comparison of the Effect of 0.5 mm Cadmium Acetate alone.

Treatments	MI	Normal Cells				Abnormal Cells						Total aberrations
		Prophase	Metaphase	Anaphase	Telophase	St. Meta	Dis.Meta	Laggards	C-Mitosis	Bridges	Fragment	
D/W	59.0b±0.52	213.0c±2.05	55.0b±2.16	61.3b±2.68	84.3a±2.33	0.00d	0.00c	0.00c	0.00d	0.00b	0.00c	0.0
Turm+D/W	78.6a±0.14	327.0a±1.19	66.0a±1.25	78.3a±1.19	65.0b±1.25	3.3c±0.72	0.00c	1.66c±0.27	8.66c±0.98	1.0a±0.001	0.00c	14.62
Cd	44.7c±0.18	207.c2±1.41	19.0c±0.72	5.3c ± 0.72	7.0d±0.94	33.6a±1.19	20.6a±0.66	25.33a±0.72	15.0b±1.41	1.33a±0.27	10.0b±0.8	105.86
Turm+Cd	22.2d±0.86	293.1b±1.91	4.0d±0.72	7.0c±0.94	15.3c±0.72	11.3b±0.98	2.0b±0.6	14.0b±0.47	20.6a±0.72	0.00b	17.0a±0.81	64.96

After the priming with 5 µg/ml turmeric extract on MI, normal and abnormal mitotic stages in the root tip cells of mung bean. Data represented mean ± SE (n = 3). Different alphabets represent significant differences among treatments at 0.05 using duncan multiple range test**.** Whereas, when seedlings were treated with cadmium alone sticky metaphase was highest (33.66%). Fig. 6 and 7 depicts pictorial representation of normal mitotic stages and abnormal cell division in mung bean root tips respectively.

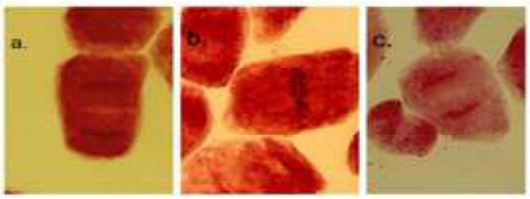

Fig. 6 Normal mitotic stages in mung bean root tips.

Furthermore Table 2 showed that MI was 59.06% in control, while it was increased to 78.66% after pretreatment of seeds with turmeric extract (5µg/mL) with few total aberrations (14.62) as compared to control (59.06%). However, in Cd treated seedlings, MI was decreased (44.75%) with increased number of aberrations (105.86). It was further noticed that cadmium treatment after pretreatment with turmeric extract, MI was reduced (22.26%) as compared to both pretreated with turmeric (5 µg/mL) prior to Cd as well as in cadmium stress alone but total aberrations were less than cadmium treated samples (64.96).The number of aberrations induced by cadmium represented the highest Geno toxicity of Cd at the rate (105.86) in mung bean roots cells and it is significantly highest when compared with un-

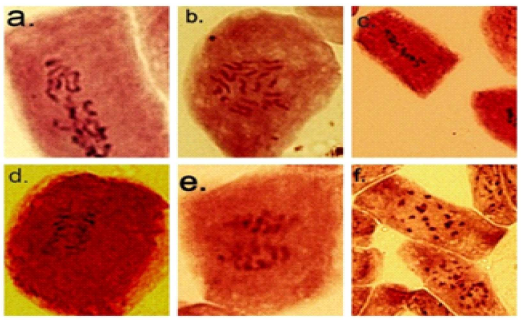

Fig. 7 Aberrant stages of mitosis in mung bean root tips.

treated control (see table 2). Total aberrations were reduced to 64.69 when cadmium treatment was given after pretreatment of 5 µg/mL turmeric extract. The most frequent aberration in Cd treatment group was sticky metaphase (33.66%) while least frequent aberration were bridges (1.33%). There was less percent for all aberrations except dislocated metaphase and fragmentation in root cells pretreated with turmeric 5 µg/mL with no cadmium with least total aberrations (14.62). Application of cadmium after the pretreatment of turmeric reduced MI and growth, however there was reduction in total aberrations as compared to cadmium treated sample (Positive control), this suggests the turmeric extract exhibited potential to reduce the division of cancerous cells without enhancing chromosomal aberrations/DNA damage action, trapping of free radicals, formation of complex with mutagens, modulation of mutagen metabolism by absorbing the xenobiotic (Premkumar *et al.,* 2004). The modulatory role of curcumin in inhibiting mutagenicity and carcinogenicity can also be due to its antioxidant activity (Nagabhushan and Bhide 1987). The biological activities of curcumin are derived from the action of the aryl group in â-diketone and the antioxidant property of the methoxy phenol group (Jovanovic *et al.,* 2001, Sun *et al.,* 2002, Anto *et al.,* 2002). In the era of increasing population, the environmental condition is getting worse specifically in the development countries. The Cd pollution has become a severe environmental problem for agricultural plants and humans. The most common genotoxic effect of Cd is stunted growth, leaf chlorosis and variation in the activities of many key enzymes in numerous metabolic pathways (Van-Assche *et al.,* 1979). The present study showed the effect of Cd leading reduction in mitotic index and plant growth in mung bean seeds and seedlings priming with or without turmeric extract. In this study, turmeric extract exhibits ant mutagenic potential against Cd induced damage in mung bean seedlings as indicated by reduced chromosomal aberrations. The protective effect of curcumin from turmeric is due to its antioxidant action, trapping of free radicals, formation of complex with mutagens, modulation of mutagen metabolism by absorbing *xenobiotic* (Premkumar *et al.,* 2004). The modulatory role of curcumin in inhibiting mutagenicity and carcinogenicity can also be due to its antioxidant activity (Nagabhushan and Bhide 1987). The biological activities of curcumin are derived from the action of the aryl group in a diketone and the antioxidant property of the methoxy phenol group (Jovanovic *et al.,* 2001, Sun *et al.,* 2002, Anto *et al.,* 2002).

3.3. Cytogenetic Analysis and Growth Parameter

The Cd affects DNA structure and chromosome composition by inducing chromosomal aberrations. Early investigation has been reported that the Cd had a toxic effect on cell division comprising C-mitosis and lagging chromosomes, anaphase bridges, and chromosome stickiness with reduction in growth and mitotic index (Liu *et al.,* 2003/ 2004). Cd inhibits cell division and cell growth by direct or indirect effects on auxin metabolism or carriers (Prasad, 1995). In this research, cells with chromosomes stickiness and fragmentation has found mostly happened due to the fact that mung bean seeds were poisoned with 0.5 mm concentration of Cd and 3 h of exposure. Anto *et al.,* (1996) reported that natural curcuminoids from turmeric are effective inhibitors of mutagenicity. Meanwhile the pretreatment with 5 µg/mL turmeric extract in control samples (D/W)

enhanced the cell division as represented in table 2, while the growth was extremely reduced when seeds were imbibed in turmeric before Cd treatment. Similarly, Table 2 exhibited that priming with turmeric was found to be successful in decreasing the aberrated cells under Cd in mung bean. This suggests, its anti-mutagenic activity (Yoysungnoen *et al.,* 2008) hence can be used for tumor treatment and could be an effective way to grow plants in Cd contaminated lands. Many highlights that complexion with curcumin reduces the toxicity of the metals and some curcumin complexes with metals like Cu^{+2}, Mn^{+2}, act as new metal-based antioxidants (Baum *et al.,* 2004; Leung *et al.,* 2013,). Turmeric could be used as herb medicine to inhibit cancerous cells produced due to metal toxicity.

In the recent years, curcumin, a compound of turmeric gained attention for the scientific researchers. Several studies have been conducted on the biological effects of curcumin, shown to have various effects in cancer treatments. Several studies suggest that curcumin can thwart the development and spread of cancer or reduce its size due to angiogenic effects and interfering with cell division (Parasad et al., 2014), and can activate cancer suppressive genes such as P53 (H-I et al., 2005) Curcumin has antibacterial, antifungal, antioxidant, antiviral, anti-inflammatory, anti-proliferative, pro-apoptotic effects. Curcumin is multifaceted molecule and has tremendous potential for treatment of neurodegenerative diseases, arthritis, diabetes, allergies, intestinal inflammation, kidney poisoning, Alzheimer's, depression, AIDS, cardiovascular disease, and especially cancer (Singh, 2005; Agarawal and Harikumar 2009). The multifaceted effects of curcumin are due to its ability to interact with different molecules, and to control multiple molecular pathways and their targets (Zhou *et al.*, 2011).

4. CONCLUSION

In current study mung bean used here was acted as a model system for human. Cadmium was known for its toxic effect on plants and human health. The priming effect of turmeric on cadmium was negative on the growth of mung bean seedlings along with reduced chromosomal aberrations thus could be used to grow plants in cadmium contaminated lands after pretreatment with turmeric, on the other hand it could be used to reduce the cancer/tumor formation as it is supported by many researchers that turmeric is used for the cure of many diseases including cancer.

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